Biochimica et Biophysica Acta 1852 (2015) 2352–2361

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Congenital Short Bowel Syndrome: from clinical and genetic diagnosis to the molecular mechanisms involved in intestinal elongation

Christine S. van der Werf [a], Danny Halim [b], Joke B.G.M. Verheij [a], Maria M. Alves [b], Robert M.W. Hofstra [b,*]

[a] Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands
[b] Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form 11 August 2015
Accepted 12 August 2015
Available online 15 August 2015

Keywords:
Congenital Short Bowel Syndrome
Development
CLMP
FLNA
Small intestine

ABSTRACT

Congenital Short Bowel Syndrome (CSBS) is a rare gastrointestinal disorder in which the mean length of the small intestine is substantially reduced when compared to its normal counterpart. Families with several affected members have been described and CSBS has been suggested to have a genetic basis. Recently, our group found mutations in *CLMP* as the cause of the recessive form of CSBS, and mutations in *FLNA* as the cause of the X-linked form of the disease. These findings have improved the quality of genetic counselling for CSBS patients and made prenatal diagnostics possible. Moreover, they provided a reliable starting point to further investigate the pathogenesis of CSBS, and to better understand the development of the small intestine. In this review, we present our current knowledge on CSBS and discuss hypotheses on how the recent genetic findings can help understand the cause of CSBS.

1. Introduction

Short bowel syndrome (SBS) refers to the sum of functional alterations that are the result of a critical reduction in the length of the intestine. In the absence of adequate treatment, SBS presents as chronic diarrhoea, chronic dehydration, malnutrition, weight loss, and nutriment and electrolyte deficiency. In most cases, SBS occurs as a result of surgical intervention for other diseases, such as necrotizing enterocolitis and intestinal atresia. However, in a small number of cases the small intestine is already shortened at birth, leading to a diagnosis of Congenital Short Bowel Syndrome (CSBS). CSBS is a heritable gastrointestinal disorder, first described by Hamilton et al. in 1969 [1]. For many years the underlying genetic cause of the disease was unknown. Recently, mutations in *CLMP* were identified to cause the autosomal recessive form of CSBS [2], and mutations in *FLNA* as the cause of the X-linked form of the disease [3]. These findings brought new insights into disease pathogenesis, but the mechanisms in which CLMP and FLNA contribute to intestinal elongation are still unknown.

In this review we describe the clinical aspects of CSBS, the recent genetic findings, and the aetiological aspects of this gastrointestinal disorder. Moreover, we hypothesise about the mechanisms underlying the development of CSBS and the signalling pathways that might be essential for development and elongation of the small intestine, based in previously described mouse models.

2. Clinical presentation

CSBS patients are characterized by the presence of a substantially shortened small intestine at birth, approximately 50 cm, when compared to 250 cm in neonates delivered at term (>35 weeks of gestation). As a consequence, they have a reduced absorptive surface of the small intestine and suffer from malabsorption [4]. CSBS can be detected by radiography, but the diagnosis is usually done by laparotomy. Patients with CSBS often present within a few days after birth with bile-stained vomiting and diarrhoea or failure to thrive, but in some cases the diagnosis has been made later in life when an exploratory laparotomy was performed for significant gastrointestinal complaint [5]. Malrotation of the bowel is always present, and although this can point to an independent developmental defect, it can also be just a consequence of the shortened small intestine. The cecum is often positioned in the left upper quadrant of the abdomen close to the splenic flexure [1,6–10], but it can also be located in the lower left quadrant of the abdomen when nonrotation of the bowel is observed [11]. In three reported patients the appendix was absent [4,7,12], and volvulus was found in four patients [13,14]. In a few cases, not only the small intestine was shortened, but also the colon was affected [4,7,12,14]. Another gastrointestinal anomaly that was described in ten CSBS patients is hypertrophic pyloric stenosis [10,13,15–18]. However, it has been suggested that hypertrophic pyloric stenosis is not part of the general developmental defect of the gastrointestinal tract, but a physiological consequence from the attempts of the remnant small intestine to slow down the gastric emptying and improve absorptive capacity. CSBS patients usually have normal intellectual ability [19,20] and do not present

* Corresponding author at: Department of Clinical Genetics, Erasmus University Medical Center, PO BOX 2040, 3000CA Rotterdam, The Netherlands.
E-mail address: r.hofstra@erasmusmc.nl (R.M.W. Hofstra).

http://dx.doi.org/10.1016/j.bbadis.2015.08.007
0925-4439/

any extra-intestinal symptoms. However, in three patients a patent ductus arteriosus was found [17,18], and in two patients minor dysmorphic features were reported [9,21].

3. Histological findings in CSBS patients

Based on the literature available, it is difficult to assess if abnormal peristalsis is associated with CSBS pathogenesis, or if it is an independent event to the presence of a short bowel [3,22,23]. In most CSBS patients the bowel wall seems macroscopically normal, but an abnormal histology has been described in some patients. Tanner et al. performed silver staining in patients' material and found an abnormally high number of neurons in the ganglia [13]. This result led the authors to suggest that the normal fall-out of ganglion cells does not occur in CSBS patients. They also found that the neuronal nuclei showed clumped chromatin, which is characteristic for neuroblasts, and the intrinsic argyrophilic ganglion cells were absent or reduced in number. However, these results lacked a quantitative analysis of the data and comparison to suitable controls to support hyperganglionosis as the cause of reduced intestinal motility. In an independent study, Schalamon et al. also observed an abnormal bowel wall with signs of neuronal intestinal dysplasia in two siblings with CSBS [2]. In another CSBS patient heterotopic gastric mucosa was found [23]. Conversely, in other cases, no abnormalities of the nerves plexus were seen on routine acetylcholinesterase staining [1,6,12,18,24–26]. Nezelof et al. described three cases with several congenital malformations, which included a shortened small intestine and heterotopia. In these patients a normal myenteric plexus was observed by cytoenzymatic and silver stainings [16]. In another study, Kapur et al. reported an extensive pathologic analysis performed on intestinal tissue collected from five male patients diagnosed with CSBS and X-linked intestinal pseudo-obstruction. They observed that these patients had diffused abnormal layering of the small intestinal smooth muscle, in which the muscularis propria layer was formed by three perpendicular muscle laminae, instead of two. Such abnormal structure was restricted to the small intestine without any extension to the colon [27]. Based on this report, a myopathic cause for the abnormal intestinal peristalsis found in CSBS patients was suggested. Despite the contradictory results, these histological findings can account for the motility abnormalities described in CSBS patients, but to date it still remains unclear whether the reduced peristalsis observed in these patients results from a neuronal or a myopathic defect.

To our knowledge, there has never been a precise histological confirmation to define which part of the small intestine is affected in CSBS patients. It is possible that every part of the small intestine is shortened in general, but one cannot rule out the possibility that only one specific part of the small intestine is affected. If this is the case, a correlation between the type of the remaining small intestine and prognosis of CSBS patients can be established. Since different parts of the small intestine have different histology and function (Fig. 1), it would not be surprising that depending on the region affected, different degrees of severity for CSBS could exist. Findings in acquired SBS cases support this idea, as it has been shown that the residual length of the jejunum and ileum with the presence of ileocecal valve (ICV), are important factors to determine the outcome of the disease [28]. Therefore, we believe that identifying the part of the small intestine that is affected in CSBS patients should be a priority.

4. Treatment management and outcome

To date, there is no cure for CSBS and patients need total parenteral nutrition for long-term survival until sufficient bowel length and functions are gained. In some cases, total parenteral nutrition has to be continued for the first two years of life [6,9], and oral feeding is introduced gradually. With time, the function of the remnant small intestine in CSBS patients improves, both in length and absorption capacity, leading to better absorption of fat and vitamin B12 [1,6,14]. The weight and height of CSBS patients are frequently below the 50th percentile [1,6,18,20,29], but no nutritional deficiencies are observed [6,9,30].

Parenteral nutrition has brought a new lease of life to an otherwise fatal condition. However, its use is often associated with very high

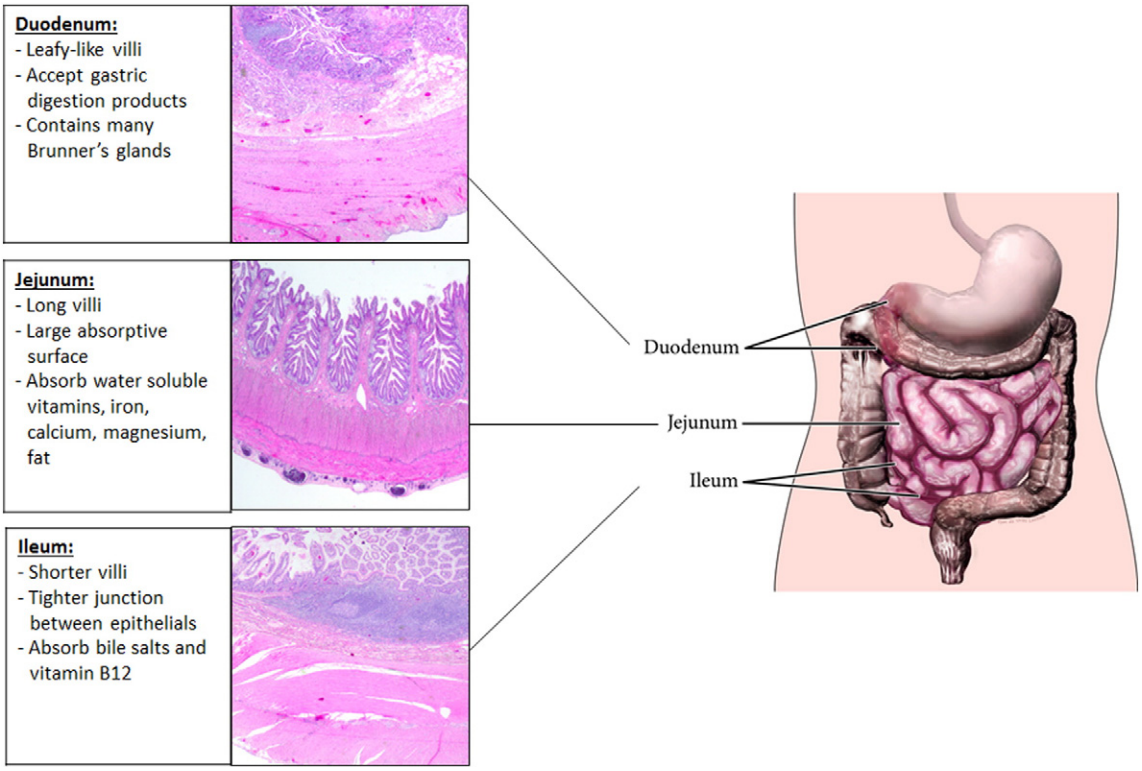

Fig. 1. Histological characteristics and function of each part of the small intestine.

rates of complications, such as sepsis and liver failure [18]. Small bowel transplantation can also be considered, but due to its relatively poor overall survival it is viewed only as a last resort treatment option. It has been recommended that CSBS patients should be managed in a multidisciplinary manner in a centre specialized in the care of children with intestinal failure [9], but despite considerable efforts to improve treatment, most patients die of starvation or sepsis within the first few days of life, and only a quarter of the reported patients survived for more than one year (Table 1).

5. Disease aetiology

To understand the causes underlying the pathogenesis of CSBS, a better understanding of the mechanisms involved in the development of the small intestine is required. In this section, we describe the embryonic events necessary for intestinal growth and elongation.

During embryogenesis, the primitive gut tube is divided in three regions: the foregut, the midgut and the hindgut, each of them with their own arterial supply (the celiac artery, the superior mesenteric artery and the inferior mesenteric artery, respectively). The small intestine (jejunum and ileum) originates from the midgut, as well as the distal duodenum, cecum, ascending colon, and the proximal two-thirds of the transverse colon. In the fifth week of embryonic development the future ileum is elongating rapidly (Fig. 2), but as the abdominal cavity grows slower, the midgut forms an anteroposterior loop called the primary intestinal loop. The cranial limb of this loop includes the ileum and the caudal limb includes the ascending and transverse colons. In the sixth week of development, the primary intestinal loop herniates into the umbilicus forced by its own elongation and growth of other abdominal organs. At this time the loop rotates 90° counter clockwise around the axis of the superior mesenteric artery. The future ileum is now lying on the right, and the cecum on the left. The cecum and the appendix continue to differentiate and the small intestine elongates further forming the jejunal–ileal loops. The attached mesentery accompanies this intestinal growth but it does so at a lower rate. Recent studies have shown that it is the differential growth rate between the intestine and the mesentery that creates specific patterns of looping and rotation of the gut in different species [31]. Therefore, any disturbance of this differential growth rate may lead to abnormal growth patterns and result in intestinal malrotation. During the tenth week of gestation, the intestinal loop returns rapidly to the abdominal cavity. The small intestine returns first and the ascending and transverse colons follow later. It is

Table 1
Overview of reported cases with CSBS as the main symptom.

Sex	Small bowel length (cm)	Reference	Year of publication	Age at time of presentation	Age at death	Familial	Consanguinity
F	40	[1]	1969	4 months	Alive at time of publication	Yes	No
F	30	[1]	1969	Unknown	1 month	Yes	No
M	30	[8]	1970	3 months	5 months	Unknown	Unknown
F	42	[14]	1973	3 days	35 days	No	No
M	70	[15]	1973	7 weeks	5 months	Unknown	Unknown
F	25	[17]	1974	1 month	21 days	Yes	Unknown
M	70	[17]	1974	Unknown	4 days	Yes	Unknown
M	45	[17]	1974	15 days	7 months	Yes	Yes
F	40	[17]	1974	5 days	2 months	No	No
M	106	[24]	1974	22 days	25 days	Yes	Yes
M	75	[7]	1976	6 days	6 months	No	No
M	70	[16]	1976	18 days	22 days	Yes	No
M	Unknown	[16]	1976	7 days	16 days	Yes	No
M	50	[16]	1976	15 days	7 months	Yes	No
M	24	[11]	1984	32 days	55 days	Yes	No
M	27	[11]	1984	2 days	5 months	Yes	No
F	45	[39]	1984	Unknown	3 months	Yes	Yes
F	45	[39]	1984	1 day	6 weeks	Yes	Yes
M	72	[18]	1984	6 weeks	Alive at time of publication	Yes	No
M	65	[18]	1984	18 days	2 months	Yes	No
M	24	[40]	1985	3 days	55 days	Yes	No
M	27	[40]	1985	2 days	5 months	Yes	No
M	45	[7]	1985	5 weeks	2 months	No	No
M	69	[6]	1986	5 weeks	Alive at time of publication	No	Yes
M	112	[42]	1990	1 month	6 weeks	Yes	No
M	70	[42]	1990	6 hours	6 months	Yes	No
M	237[a]	[42]	1990	3 months	Alive at time of publication	Yes	No
F	54	[19]	1991	2 months	Alive at time of publication	No	No
F	30	[26]	1993	2 days	4 months	Yes	No
M	39	[26]	1993	Unknown	6 months	Yes	No
F	30	[26]	1993	Unknown	2 months	No	No
F	30	[43]	1996	1 day	Alive at time of publication	No	No
F	50	[21]	1997	1 day	Alive at time of publication	No	No
F	25	[12]	1998	4 days	6 months	No	No
M	47	[20]	1999	9 days	Alive at time of publication	Yes	Yes
M	42	[25]	2001	2 days	5 months	Yes	No
F	51	[25]	2001	3 days	2 weeks	Yes	No
M	95	[25]	2001	2 months	Alive at time of publication	No	Yes
M	35	[25]	2001	2 days	2 months	Yes	No
M	228.6[b]	[5]	2002	15 years	Alive at time of publication	No	No
M	56	[4]	2004	4 months	Alive at time of publication	No	No
F	20	[29]	2004	4 days	Alive at time of publication	No	No
F	30	[9]	2006	5 days	Alive at time of publication	Yes	No
M	50	[30]	2008	6 weeks	Alive at time of publication	Yes	Unknown
M	20–25	[44]	2010	26 days	1 month	No	No
M	Unknown	[3]	2013	Unknown	Alive at time of publication	Yes	No

[a] At 14 years of age.
[b] At 15 years of age.

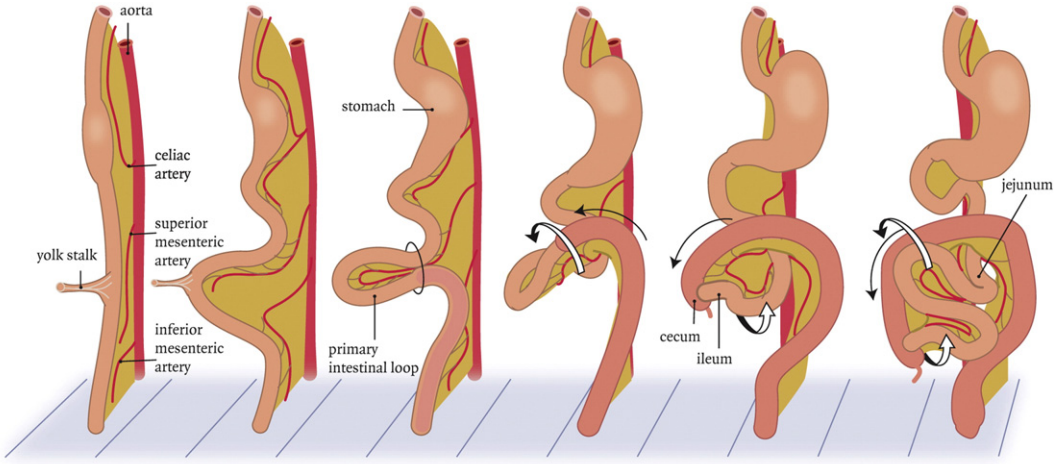

Fig. 2. Embryogenesis of the human small intestine.

not known what causes this retraction, but an increase in the size of the abdominal cavity and a relatively decrease in the size of the liver and kidneys seem to play an important role. To reach the definitive configuration of the small and large intestines, the intestinal loop rotates another 180° counter-clockwise [32–34].

Following this intricate growth, looping and rotation of the gut, intestinal patterning and regionalization takes place. All intestinal organs derived from the foregut, midgut and hindgut consist of similar layers, which include mucosa, muscularis mucosa, submucosa, submucosal plexus, muscularis propria, myenteric plexus and serosal layer. Formation of these layers requires extensive proliferation and differentiation of cells that compose the epithelial and muscle of the intestine. The neuronal network responsible for intestinal innervation is mainly formed by enteric neural crest cells (ENCCs) that migrate from the neural tube in a rostral to caudal direction to promote neuronal colonization of the intestinal tract [35]. Interestingly, cell differentiation and radial patterning of the gut are largely dictated by signals exchanged between the different cell types that constitute the various layers. For example, the hedgehog signalling initiated in the intestinal epithelium affects cell proliferation and differentiation of cells derived from all embryonic layers (endoderm, mesoderm and ectoderm), playing an important role in the establishment of the villus-crypt patterning, smooth muscle cells' proliferation, and ENCCs' proliferation, differentiation and migration [36,37]. Several other morphogenic pathways regulate cellular interactions and are thus, necessary for intestinal elongation. These pathways are discussed further in Section 8 of this review. Nonetheless, it is important to acknowledge here the existence of such interactions, since the gut abnormalities found in CSBS patients may well arise due to dysregulation of these morphogenic pathways.

6. Genetics

Familial occurrence of CSBS was described in the very first case report in 1969. Hamilton et al. reported a French-Canadian couple who were not related and who had five daughters, two of them diagnosed with CSBS. One of the affected girls died at the age of 1 month and 1 week, and prior to death a laparotomy showed a small intestine of 30 cm in length [1]. More case reports followed and a familial occurrence was described in approximately 60% of the cases published in the literature. In most of these cases siblings were affected, and in approximately 25% of the cases the parents were consanguineous (Table 1). It has therefore been suggested by several authors that genetic factors were involved in CSBS and an autosomal recessive pattern of inheritance was proposed by many of them [6,10,17,19,25,38–40]. However, since only boys were found affected in some families, an X-linked pattern of inheritance has also been suggested [16,18,41,42]. In this section, we focus on the genetic findings described in CSBS patients.

6.1. Chromosomal abnormalities

Two patients have been described with chromosomal abnormalities. Hou et al. reported a female patient with multiple congenital anomalies, such as congenital short bowel, malrotation, and patent ductus arteriosus, in addition to major malformations, such as left upper amelia, dextrocardia and asplenia. Chromosomal investigation of this patient showed a mosaic pattern with complex rearrangements of chromosome 4: 85% of the peripheral lymphocytes showed a normal female cell line (46,XX), while 12% of the cells showed a pattern with one normal chromosome 4 and a ring chromosome 4 (46,XX-4,+r(4)(p16➔q22.3). The ring caused a deletion of the long arm of chromosome 4. Approximately 4% of the cells from this patient had a pattern with partial trisomy of chromosome 4: one normal chromosome 4, one ring chromosome 4 and one chromosome 4 with the same deletion of the long arm of chromosome 4 (47,XX,4,+r(4)(p16➔q22.3),+del(4)(pter➔q22.3:)) [43]. De Backer et al. also described a female CSBS patient with a *de novo* balanced translocation between chromosome 2 and 11 (46,XX,t(2,11)(q32.2,p12)) [21]. However, no functional implications of these chromosomal abnormalities have been reported in these cases that could explain the development of CSBS.

6.2. Loss-of-function mutations in CLMP cause recessive CSBS

In seven CSBS patients from five unrelated families, different homozygous and compound heterozygous loss-of-function mutations have been identified in the gene encoding for the Coxsackie- and adenovirus receptor-like membrane protein (CLMP) [2]. The reported length of the small intestine of these patients was 30 to 54 cm and they all presented malrotation of the bowel. Neuronal intestinal dysplasia was reported in two patients (from one family) with a more complex mutation, presumably an inversion [2,20].

CLMP, located on chromosome 11 (11q24.1), encodes a transmembrane protein that co-localizes with tight junction proteins and acts as an adhesion molecule [2,45]. It is expressed in the intestine during different stages of human development, and knockout of its orthologue in zebrafish resulted in developmental defects of several organs, including the intestine. As tight junction proteins play an important role in proliferation [22,46], we hypothesised that loss-of-function of CLMP results in less proliferation of the small intestinal cells during human development, leading to a shortened small intestine at birth [2]. Recently, this hypothesis was tested using an *in vitro* approach where a mutant CLMP (V124D), was over-expressed in a human intestinal epithelial

cell line (T84). This mutant has been previously reported to mislocalize to the cytoplasm [2], but *in vitro* assays failed to confirm the role of CLMP in cell viability, proliferation and migration in this cell line [47]. Thus, the role of CLMP in small intestinal development and its function still remain unclear.

6.3. Mutations in FLNA cause X-linked CSBS

Mutations in Filamin A (*FLNA*) have been associated with a wide spectrum of disorders characterized by a variable phenotype. Loss-of-function mutations are found in patients with bilateral periventricular nodular heterotopia, a neuronal migration disorder characterized by seizures affecting mainly females, as it is often lethal in males [48]. Mutations that alter the function of *FLNA* are associated with three different disorders: otopalatodigital syndromes type 1 and 2, frontometaphyseal dysplasia, and Melnick Needles syndrome. These syndromes constitute a phenotypic spectrum that includes skeletal dysplasia, craniofacial-, cardiac-, genito-urinary and intestinal anomalies, and central nervous system defects [49]. In addition, missense mutations in *FLNA* are associated with X-linked cardiac valvular dystrophy [50,51]. CSBS has also been described in some of the patients reported with loss-of-function mutations in *FLNA*. However, these patients presented multiple congenital anomalies and the short bowel was described as part of the disease phenotype. A male patient, stillborn at 33 weeks of gestation, was reported with a duplication of the first 28 exons of *FLNA*. Prenatal ultrasounds showed normal growth with a single umbilical artery, umbilical vein varix, and persistent dilatation of the bowel first seen at 20 week gestation. These findings were confirmed by an autopsy of the foetus, which also detected a bifid uvula, an atrial septal defect, and a malrotated short small intestine of 45 cm (164 cm would be the expected length at this gestational stage). This duplication was also identified in his mother, who was diagnosed with a bifid uvula and patent ductus arteriosus, and in his maternal uncle, who had multiple congenital anomalies including a bifid uvula, intestinal malrotation, undescended testes, partial agenesis of the corpus callosum, patent ductus arteriosus, patent foramen ovale, ventricular septal defect and periventricular heterotopia, and a small intestine measuring only 115 cm at the age of 10 years [27]. Another patient with a hemizygous nonsense mutation in *FLNA* (c.7021C>T, Q2341X) was diagnosed prenatally with a left diaphragmatic defect, which caused a displacement of the spleen, left hepatic lobe, and portions of the stomach and small intestine into the left hemithorax. He also had dysmorphic facial features, spina bifida occulta, natal tooth, periventricular heterotopia, a posterior fossa arachnoid cyst, and proximally placed thumbs. He died at 6 weeks of age and his small intestine measured only 68 cm [27]. Recently, a family has been described with two affected male siblings where a novel no-stop mutation in *FLNA* (c.7941_7942delCT, p.(*2648Serext*100)) was identified upon genetic screening. The same mutation was detected in their male cousin. These patients were diagnosed with CSBS, “wandering spleen”, periventricular nodular heterotopia, persistent ductus arteriosus, and urinary tract abnormalities [52]. All these reports confirm that CSBS patients with mutations in *FLNA* have, in general, multiple congenital anomalies in addition to a shortened small intestine. Recently, however, a mutation in the second exon of *FLNA* has been identified in three male patients (from two different families) where CSBS appeared as an isolated symptom without other major congenital anomalies [3]. In these patients, a two-base-pair (bp) deletion in *FLNA* (c.16–17delCT) was identified. In another male patient previously described with Chronic Idiopathic Intestinal Pseudo-obstruction, a 2-bp deletion was also found in the second exon of *FLNA* (c.65–66delAC). This patient was diagnosed with malrotation, pyloric hypertrophy, intestinal pseudo-obstruction, and CSBS [53,54]. In these four patients the length of the small intestine ranged from 55 to 235 cm, and the age of diagnosis varied from 1 day to 15 years [5,15,42], suggesting that in some cases, the small intestine is less reduced in length and the diagnosis is made later in life when compared to CSBS patients with *CLMP* mutations. These 2-bp deletions are located between two nearby methionines at the N-terminus of *FLNA*. Previous studies showed that translation of FLNA occurs from both methionines, resulting in two protein isoforms [54]. In the presence of this 2-bp deletion the longer isoform is not translated anymore, but there is still expression of the shorter FLNA isoform. We hypothesised that this is the reason why these deletions are not lethal for males *in utero*, and they only develop CSBS. Screening of exon 2 of *FLNA* is therefore, recommended in such cases. In X-linked families where CSBS is associated with multiple congenital anomalies it is advisable to screen the entire *FLNA* for mutations.

FLNA encodes a cytoskeletal protein that binds to actin and has a well-characterized role in the cytoplasm. It regulates cell shape by cross-linking actin filaments, and plays an important role in cell signalling and migration in response to environmental changes [55]. A role for FLNA has also been recently discovered in the nucleoli, where it inhibits ribosomal RNA transcription [56]. FLNA has been reported to play an important role in vascular development and cardiac morphogenesis [57], but its role is still unclear in intestinal development. Nishita et al. reported that FLNA is able to interact with the tyrosine kinase-like orphan receptor 2 (Ror2), and showed that this interaction is required for filopodia formation and migration [58]. Since disruption of Ror2 expression has been shown to lead to a shortened small intestine in mice [59], it is tempting to hypothesise that *FLNA* mutations leading to CSBS disrupt the FLNA–Ror2 interaction and impair cell migration. However, further studies are required to clarify the role of FLNA in intestinal elongation.

7. Link between CLMP, FLNA and CSBS

We now know that mutations in *CLMP* and *FLNA* underlie CSBS pathology [2,3]. However, it is still unclear whether CLMP and FLNA interact with each other (directly or indirectly) and, therefore, whether mutations in one of these two genes result in a similar course of events during development of the small intestine. In this section, we discuss different hypotheses to explain CSBS pathogenesis, and speculate about a possible link between CLMP, FLNA and each of these hypotheses.

7.1. Embryonic and intrauterine events

As mentioned in Section 5, during the seventh and tenth weeks of embryonic development, the primitive digestive tube needs to return to the intraumbilical coelom. Hamilton et al. suggested that in CSBS this process is prevented. As a consequence, the primitive bowel is forced to stay in the abdominal cavity and the cranial portion of the bowel is not able to elongate, leading to a shortened small intestine [1]. In another report, Aviram et al. observed the presence of bowel loops inside the umbilical cord on a prenatal sonography of a patient with CSBS, showing that the gut was able to elongate, but the return of the intestine to the abdominal cavity was in fact delayed. They speculated that the incomplete dextral rotation and elongation of the bowel caused this delay [38]. Delayed return of the intestine to the abdominal cavity is also associated with volvulus and intestinal obstruction [60]. However, volvulus has only been described in four CSBS cases, and adhesions, atresia, stenosis and scars are rarely found in CSBS patients. In a case report of a premature neonate with an absent small bowel, there was also no evidence of an abdominal wall defect or other intra-abdominal anomalies on prenatal sonography [61]. Antenatal intussusception followed by auto-anastomosis and auto-amputation is also suggested as a cause of CSBS [12]. However, failure of intestinal elongation can also be the cause, rather than the outcome of the observed malrotation in CSBS patients [15]. In cases were auto-anastomoses were found, intrauterine events like volvulus and infarction can be a reasonable explanation for the shortened small intestine [4]. Another hypothesis is that vascular events underlie CSBS [15]. Intrauterine

infarction of the bowel may lead to reabsorption of the ischemic bowel and result in a decreased length of the remaining gut.

Considering the proposed embryologic and intrauterine events one could hypothesise that CLMP might play a role in these events. As mentioned earlier, tight junction proteins play an important role in proliferation [22,46]. Hence, we speculate that loss-of-function of CLMP results in less proliferation of the small intestinal cells during human development, leading to a shortened small intestine at birth. The processes and pathways that may be disturbed by the loss of CLMP still remain unknown.

FLNA can also be linked to embryologic and intrauterine events, since it is known to play an important role in vascular development. Patients with *FLNA* mutations have been reported with developmental anomalies of the blood vessels [62,63], and omphalocoele [55]. An intrauterine vascular event causing small intestinal infarction [15] might, therefore, be a reasonable explanation for the development of CSBS, supporting the hypothesis that the developmental defect seen in CSBS patients originates in the embryonic stage at which the bowel is accommodated in the intraumbilical coelom [1].

7.2. Lack of neurotransmitters and hormones

As abnormal peristalsis has been observed in some CSBS patients, Sansaricq et al. hypothesised that these patients may lack synthesis of neurotransmitters [18]. However, abnormal peristalsis is not described in all patients. In another report, Schalamon et al. suggested that CSBS patients lack growth-stimulating hormones like epidermal growth factor, insulin-like growth factor, and human growth hormone, but they were unable to detect abnormal hormone levels in their patients [20].

How mutations in *CLMP* and *FLNA* can lead to disturbed or lack of neurotransmitters is unclear. However, as these genes do not encode or even have an (direct) effect on the production of neurotransmitters, it is unlikely that a lack of neurotransmitters is the cause for the shortened small intestine observed in CSBS patients.

7.3. CLMP and FLNA

CSBS patients with mutations in *CLMP* seem to have a phenotype more restricted to the intestine, whereas patients with mutations in *FLNA* are more likely to have multiple congenital anomalies. However, CSBS patients with a deletion in the second exon of *FLNA* are very similar to patients with mutations in *CLMP*. This observation together with the fact that both gene products are involved in similar cellular processes, such as cell–cell contact and actin organisation, suggest that FLNA and CLMP might be involved in the same protein network that is essential for intestinal development.

FLNA is an actin-binding protein and its actin-binding domain is located in its N-terminal region [55,64,65]. Gargiulo et al. showed abnormal actin organisation in a lymphoblastoid cell line of a CSBS patient with a c.65–66delAC deletion in *FLNA* [53]. Therefore, there is evidence that the cytoskeletal actin organisation is disturbed in CSBS patients with mutations in *FLNA*. However, it is still not known whether patients with mutations in *CLMP* have a similar problem. Raschperger et al. showed that CLMP co-localizes with actin filaments [45]. They speculated that CLMP interacts with a protein that directly binds to actin filaments, which would bring CLMP to the tight junctions by anchoring CLMP to the actin cytoskeleton. They suggested that ZO-1 could be such an interacting protein. As FLNA also binds to actin filaments and is known to play a role in anchoring transmembrane proteins to the cytoskeleton for correct targeting to the cell membrane, such as integrin beta and the cystic fibrosis trans-membrane conductance regulator (CFTR) [65,66], it is tempting to suggest that FLNA is the link between CLMP and the actin cytoskeleton and is responsible for proper localization of CLMP to the tight junctions (Fig. 3). Another possibility is that FLNA plays a role in the internalization of CLMP into the plasma membrane. As FLNA is known to control the internalization of the chemokine receptor 2B in different dynamic membrane structures [67], one can speculate that mutations in *FLNA* influence the expression levels of CLMP on the plasma membrane. Further research is needed to determine whether CLMP and FLNA interact with each other as part of the same protein network and, if so, which other proteins are involved in this network. However, we cannot exclude the possibility that different pathways underlie X-linked CSBS and autosomal recessive CSBS, with different disease mechanisms leading to a similar disease phenotype.

It is also not known whether more genes are involved in the pathogenesis of CSBS. We did not identify a mutation in all patients screened for *CLMP* and the second exon of *FLNA*. However, we did not screen all exons of *FLNA* in all patients analysed, which means that FLNA might still play a role in disease development in some of these patients. Based on the finding of an abnormal karyotype with a ring chromosome 4 in one CSBS patient with multiple congenital anomalies [43], a gene on the long arm of chromosome 4 might also be involved. Further research on the protein networks of CLMP and FLNA might help find more candidate genes for CSBS.

8. Mouse models for CSBS and intestinal elongation

In order to get new insights about the pathogenesis of CSBS, observations on *CLMP* and *FLNA* knockout animal models might be important. They can help identify which cell types are affected, and also determine if there is a general shortening or if specific parts of the intestine are affected. To date, there is no mouse model available for *CLMP*, only a zebrafish model that was recently reported by our group [2]. In this model, there was an overall reduction of the body length accompanied by a drastic reduction in the size of the small intestine. Histological findings showed that there was also a significant difference in gut morphology in the mutant fish, marked by the absence of goblet cells in the mid intestine. This result confirmed the importance of CLMP for small intestine development and elongation, but further investigation is required to determine the role of CLMP in intestinal embryogenesis.

For *FLNA*, two mouse models have been reported: a conditional knockout model, and a N-ethyl-N-nitrosourea induced model called *Dilp2* [57,68]. Both of them showed complete loss of *Flna* and resulted in embryonic lethality. Vascular defects were also reported in both of these models, and in one of them there was a delayed resorption of the umbilical hernia [68]. Feng et al. further investigated the reasons associated to the vascular phenotype, and showed that migration and motility of different cell types were not affected in *Flna*-null embryos [57]. However, abnormal epithelial and endothelial organisation, and aberrant adherent junctions were observed in several tissues, including the developing blood vessels, heart, and brain. A defect in intestinal elongation was not reported in any of these models, but it is possible that a more detailed examination of these embryos could shed some light into the role of FLNA in normal intestinal development.

With respect to intestinal elongation, several mouse models have been reported with a shortened small intestine: *Fgf9*$^{-/-}$; *Shh*$^{-/-}$ *Ihh*$^{-/-}$; *Notch*$^{-/-}$; *Wnt5a*$^{-/-}$; *Ror2*$^{-/-}$; *Sfrp1*$^{-/-}$*Sfrp2*$^{-/-}$*Sfrp5*$^{-/-}$ and *Hlx*$^{-/-}$ [59,69–74]. All these genes encode for proteins involved in highly conserved signalling pathways known to play a crucial role in normal embryonic development [69]. In this section, we describe these proteins and associated pathways, and discuss their contribution towards the elongation of the small intestine.

8.1. Fibroblast Growth Factor 9 (FGF9)

FGF9, also known as glia activating factor, is part of a large family of polypeptide growth factors that are involved in a variety of biological processes, such as embryonic development, cell growth, morphogenesis, tissue repair, tumour growth and invasion [75]. A knockout mouse model for *Fgf9* showed a disproportional small intestine, suggesting that Fgf9 is particularly important for small intestinal morphogenesis. However, this effect was only seen after embryonic day 14.5 (E14.5),

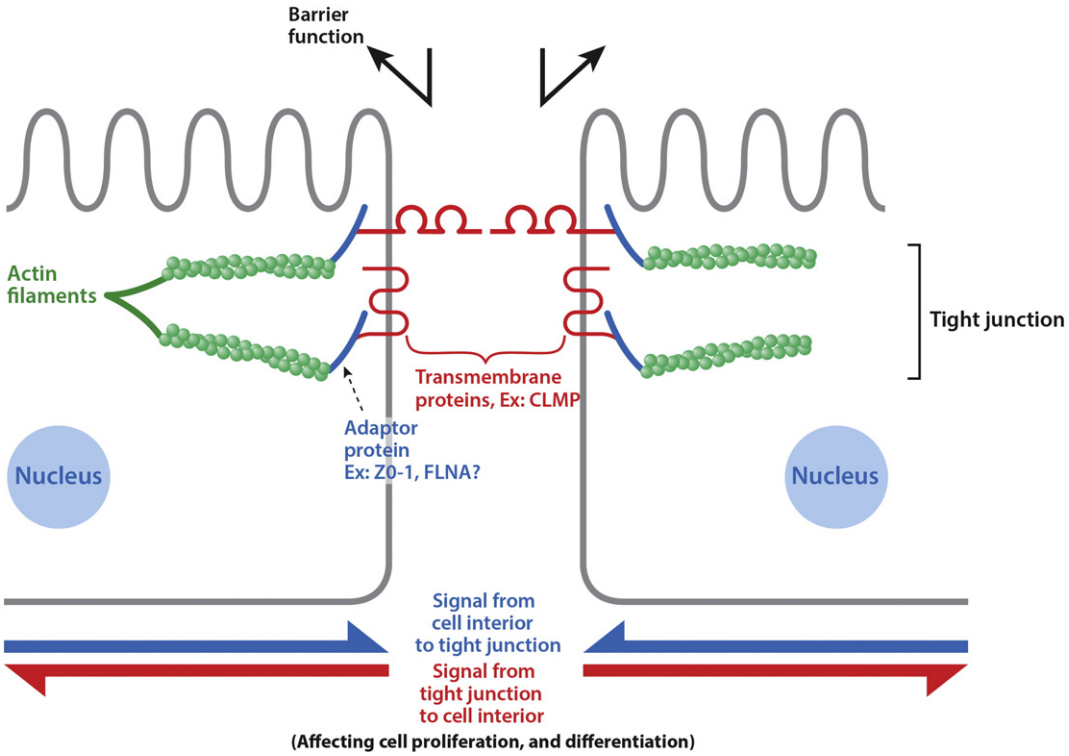

Fig. 3. Tight junction complexes and the possible link between CLMP and FLNA.

suggesting that Fgf9 regulates small intestinal elongation during late stage embryogenesis [69]. The mechanism by which FGF9 regulates this process is not totally known, but Geske et al. showed that this effect might be due to a significant increase of the transforming growth factor beta (TGFβ) signalling pathway in the absence of FGF9 [69]. TGFβ signals are well-known to drive the transition of mesenchymal fibroblasts to myoblasts by activation of intermediate molecules, such as Smad2 and Smad3 [76]. However, this process is mostly seen as the small intestine transitions to the postnatal period, when the proliferative properties of the mesenchymal fibroblasts are less required. In the *Fgf9*$^{-/-}$ mouse model, an increase in TGFβ signalling was detected at earlier stages of embryogenesis, leading to a premature differentiation of the mesenchymal fibroblasts into myofibroblasts and hence, to decreased proliferation [69]. As a consequence, the normal elongation of the small intestine was impaired.

8.2. Sonic (Shh) and Indian hedgehog (Ihh)

The mammalian family of lipid-modified hedgehog (Hh) signals are composed of three members: Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh). Each of them is thought to signal through a common mechanism that involves binding and inactivation of Ptch1, a multi-pass transmembrane receptor. As a consequence, the seven-pass membrane protein smoothened (Smo) is activated, leading to a transcriptional response controlled by the Gli family of transcription factors [77]. Hh signalling is involved in proliferation, patterning and differentiation of many tissues [78]. In the mammalian gut, Shh and Ihh are known to be co-expressed in the endodermal epithelium from early developmental stages (E8.5 of mouse development) [79], and dysregulation of this pathway has already been implicated in both congenital defects and cancers arising from the gastrointestinal tract [80,81]. However, mouse models where the expression of *Shh* or *Ihh* was abolished only showed limited anomalies, likely due to functional redundancy between these two proteins [77]. Mao et al. generated a double mutant mouse model where expression of both *Shh* and *Ihh* was blocked [70]. They showed that at E11.5 the digestive tract of double mutant embryos was normal in shape, orientation and location in the embryo, but was dramatically reduced in size relative to wild-type controls. At the end of gestation this difference was even more striking, as the intestine of *Shh* and *Ihh* null embryos completely failed to expand [70]. However, the primary patterning of the gut into distinct organ segments was not affected. They further investigated the reasons behind the phenotype and showed that double null embryos present a dramatically reduced number of mesenchymal progenitors necessary for normal endodermal–mesenchymal interplay in the mammalian gut. Vasculature integrity was intact and no significant change in the levels of necrotic and apoptotic markers were detected in these embryos, suggesting that the decreased number of mesenchymal progenitors was a primary effect of absent Hh signalling. Considering that intestinal smooth muscle cells are derived from local mesenchymal progenitors, the authors also investigated the effect of absent expression of Shh and Ihh for smooth muscle development [70]. They showed that double mutant embryos do not express smooth muscle α actin (SMA), and have impaired smooth muscle differentiation due to reduced expansion of the early mesenchymal progenitor pool. Taken together, these results showed that Hh signalling is a mitogenic factor necessary for expansion of gut mesenchymal progenitors including those of the smooth muscle compartment, and is thus, essential for embryonic gut development.

8.3. Notch

The *Notch* gene encodes for a transmembrane receptor protein known to activate a signalling cascade critical for normal embryonic development and tissue homeostasis [82]. In mammals there are four Notch receptors (Notch 1–4), which upon ligand binding, trigger a series of proteolytic cleavages to release the intracellular domain of Notch (NICD), a biologically active signal transducer [83]. NICD translocates to the nucleus and binds to a transcription factor, recombining binding protein suppressor of hairless (RBP-J), and to a co-activator, Mastermind, activating transcription of several target genes involved in cell proliferation or apoptosis [84]. The Notch signalling pathway is widely used in different cell types and cellular processes. Therefore, it

is not surprising that defects in this pathway are associated with developmental disorders and cancers [85,86].

In the intestine, genetic analyses of zebrafish and mouse mutants have revealed a requirement of the Notch signalling pathway for cell expansion and proper lineage allocation of epithelial progenitors [87,88]. In addition, this pathway is particularly active in the developing intestinal mesenchyme, specifically in sub-epithelial fibroblasts, and its dysregulation was shown to play a role in intestinal elongation. Selective disruption of the Notch pathway in the mesenchyme by the use of a conditional knockout mouse for the effector gene *Rbpj*, or constitutive activity of Notch by forced expression of NICD, led to a reduction of intestinal length [71]. In both cases, a progressive loss of sub-epithelial fibroblasts was detected, but the phenotype observed was more severe in the presence of constitutive Notch activation. A close inspection of the mutant embryos showed that despite similarities in the phenotype, the mechanisms leading to a reduction of sub-epithelial fibroblasts were different in each case. While the absence of Notch signalling led to reduced proliferation of sub-epithelial fibroblasts but no significant increase in apoptosis, over-activation of this pathway resulted in apoptosis, and consequently cell death [71]. These observations showed that the Notch signalling plays a critical role in the development of the intestinal mesenchyme, and revealed that tight regulation of this pathway is needed to fine-tune its effects during intestinal elongation.

8.4. Wnt5a

The Wnt proteins are secreted glycoproteins known to activate various intracellular signalling cascades upon binding to their receptors, Frizzled (Fzd) and/or transmembrane co-receptors, such as the lipoprotein receptor-related protein 5/6 (Lrp5/6), Ror2, and the related to receptor tyrosine kinase (Ryk) [89]. Wnt signalling pathway is divided in two general categories – canonical and non-canonical – based on transcriptional involvement of β-catenin. Independent of the pathway activated, Wnt signalling is essential for diverse processes, including cell fate, proliferation, differentiation, migration, polarity and asymmetric cell division [90].

Wnt5a is one of the ligands involved in the activation of the non-canonical Wnt signalling pathway. It is known to bind Ror2 and is required for normal embryogenesis, playing a pivotal role in the elongation process of several organs, including the small intestine [72,91,92]. Loss of either Wnt5a or Ror2 expression in mice was reported to lead to a dramatic shortening of the small intestine [59,72]. Accordingly, expression levels of Wnt5a and Ror2 have been shown to peak during the critical period of midgut elongation in mice, which is between E10.5 and E13.5 [93]. Moreover, Bakker et al. showed that Wnt5a expression during mouse intestinal embryogenesis is tightly orchestrated in certain time frames, and overexpression or loss of expression during the critical period of midgut elongation (before E13.5) leads to intestinal elongation defects [93]. It is still not clear how Wnt5a and Ror2 regulate intestinal elongation, but at the cellular level, they are known to be involved in cell migration and proliferation [94]. Therefore, a decrease in cell proliferation and migration induced by the absence of Wnt5a or Ror2, might lead to a shortened small intestine.

Wnt5a is also known to activate the Wnt/Jun N Kinase (JNK) signalling pathway, defined as the planar cell polarity pathway, as it mediates orientation of the cell movements during development [95]. Previous studies showed that JNK plays an irreplaceable role in preserving endoderm cell–cell adhesion and maintaining the stability of microtubules, which are required for normal intestinal elongation. However, activation of this pathway has to be tightly regulated for proper GI tract development. The secreted Frizzled-related protein 1 (Sfrp1) has been shown to directly interact with Wnt5a, playing a key role regulating its activity [73]. Sfrp1 together with Sfrp2 and Sfrp5, belong to the type 1 subfamily of Sfrp antagonists of the Wnt signalling [96]. Since there is functional redundancy between the members of each subfamily [97], loss of Sfrp1, 2 and 5 in a compound mutant mouse model ($Sfrp1^{-/-}$ $Sfrp2^{-/-}$ $Sfrp5^{+/-}$) led to dysregulation of the Wnt5a signalling pathway detected by elevated levels of phosphorylated c-Jun in the epithelium of the small intestine. As a consequence, mutant embryos exhibited a shorten body axis and unsurprisingly, a dramatic reduction in gut length [73]. On the other hand, the absence of active JNK signalling led to dissociation of endoderm cells and perturbation of the cytoskeleton due to microtubule destabilisation, resulting as well, in an impairment of gut elongation [98].

8.5. HLX

The divergent murine homeo box gene *Hlx* encodes for a transcription factor that during embryogenesis is mainly expressed in tissues of mesodermal origin, such as visceral mesenchyme, skeletal myoblasts, sclerotome and limb mesenchyme [99,100]. *Hlx* expression is detected during mouse development at around E9.5 in the midgut and hindgut, and from E10.5 to E12.5 in the liver, gall bladder, and gut. Homozygous disruption of *Hlx* led to a dramatic impair of visceral organogenesis specifically of the liver (only reached 3% of its normal size) and the gut (only reached a quarter of its normal length), suggesting that neither of these organs were able to go through the dramatic expansion characteristic of normal organogenesis in the absence of Hlx [74]. A detailed examination of the $Hlx^{-/-}$ embryos showed a normal appearance with characteristic midgut and hindgut structures at E10.5. However, from E11.5 to E14.5 the extensive looping and midgut umbilical hernia observed in wild-type embryos was absent in the mutants. The $Hlx^{-/-}$ embryos exhibited only a single intestinal loop at E13.5 and E14.5, but the mesenchyme became normally stratified into histologically distinct layers [74]. The mechanism by which Hlx controls visceral organogenesis is still not well understood. Henscht et al. suggested that Hlx controls a mesenchymal–epithelial interaction critical for liver and gut extension. This interaction is likely mediated by mitogenic factors or matrix components secreted by the mesenchyme, and is required for proliferation of both liver and gut epithelia [74].

9. Concluding remarks

Genetic studies have identified two genes, *CLMP* and *FLNA*, that when mutated lead to the development of CSBS. These findings have improved the quality of genetic counselling for CSBS patients and made prenatal diagnostics possible. However, the mechanisms by which CLMP and FLNA lead to CSBS are still unknown. Mouse models have also identified several other genes that are instrumental for intestinal elongation. All these genes encode for proteins that play an instrumental role in major signalling pathways required for embryonic development. However, the involvement of these genes in CSBS has not yet been reported. Based on our current knowledge, it is difficult to place CLMP and FLNA in one of the complex networks involved in intestinal elongation, but it is tempting to speculate that CLMP and FLNA can either control or be controlled by one (or more) of these signal transduction pathways. All these findings provide new insights into CSBS pathogenesis, and represent a first step to identify major processes required for intestinal development and elongation.

Conflict of interest

There are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

The authors would like to thank Tom de Vries Lentsch for making the illustrations, and Dr. Michael Doukas for his contribution to Fig. 1. This work was supported by the Junior Scientific Masterclass (University of Groningen), the Ter Meulen Fund, the van Walree Fund, the Royal Netherlands Academy of Arts and Sciences, the Dutch Digestive Foundation, the J.K. de Cock Stichting, and the Stichting Simonsfonds.

References

[1] J.R. Hamilton, B.J. Reilly, R. Morecki, Short small intestine associated with malrotation: a newly described congenital cause of intestinal malabsorption, Gastroenterology 56 (1969) 124–136.
[2] C.S. Van Der Werf, T.D. Wabbersen, N.H. Hsiao, J. Paredes, H.C. Etchevers, P.M. Kroisel, D. Tibboel, C. Babarit, R.A. Schreiber, E.J. Hoffenberg, M. Vekemans, S.L. Zeder, I. Ceccherini, S. Lyonnet, A.S. Ribeiro, R. Seruca, G.J. Te Meerman, S.C. van Ijzendoorn, I.T. Shepherd, J.B. Verheij, R.M. Hofstra, CLMP is required for intestinal development, and loss-of-function mutations cause congenital short-bowel syndrome, Gastroenterology 142 (2012) 453–462 (e453).
[3] C.S. van der Werf, Y. Sribudiani, J.B. Verheij, M. Carroll, E. O'Loughlin, C.H. Chen, A.S. Brooks, M.K. Liszewski, J.P. Atkinson, R.M. Hofstra, Congenital short bowel syndrome as the presenting symptom in male patients with FLNA mutations, Genet. Med. 15 (2013) 310–313.
[4] G. Sabharwal, P.J. Strouse, S. Islam, N. Zoubi, Congenital short-gut syndrome, Pediatr. Radiol. 34 (2004) 424–427.
[5] C. Siva, R. Brasington, W. Totty, A. Sotelo, J. Atkinson, Synovial lipomatosis (lipoma arborescens) affecting multiple joints in a patient with congenital short bowel syndrome, J. Rheumatol. 29 (2002) 1088–1092.
[6] S.F. Dorney, W.J. Byrne, M.E. Ament, Case of congenital short small intestine: survival with use of long-term parenteral feeding, Pediatrics 77 (1986) 386–389.
[7] N. Iwai, J. Yanagihara, T. Tsuto, H. Taniguchi, T. Takahashi, Congenital short small bowel with malrotation in a neonate, Z. Kinderchir. 40 (1985) 371–373.
[8] C.W. Konvolinka, Congenital short small intestine: a rare occurrence, J. Pediatr. Surg. 5 (1970) 574.
[9] P. Ordonez, J.M. Sondheimer, S. Fidanza, G. Wilkening, E.J. Hoffenberg, Long-term outcome of a patient with congenital short bowel syndrome, J. Pediatr. Gastroenterol. Nutr. 42 (2006) 576–580.
[10] N. Schnoy, G. Bein, K. Dumke, Familial occurrence of congenital short bowel (author's transl), Monatsschr. Kinderheilkd. 126 (1978) 431–435.
[11] C.M. Tiu, Y.H. Chou, H.B. Pan, T. Chang, Congenital short bowel, Pediatr. Radiol. 14 (1984) 343–345.
[12] N. Sarimurat, S. Celayir, M. Elicevik, S. Dervisoglu, D. Yeker, Congenital short bowel syndrome associated with appendiceal agenesis and functional intestinal obstruction, J. Pediatr. Surg. 33 (1998) 666–667.
[13] M.S. Tanner, B. Smith, J.K. Lloyd, Functional intestinal obstruction due to deficiency of argyrophil neurones in the myenteric plexus. Familial syndrome presenting with short small bowel, malrotation, and pyloric hypertrophy, Arch. Dis. Child. 51 (1976) 837–841.
[14] C. Yutani, M. Sakurai, T. Miyaji, M. Okuno, Congenital short intestine. A case report and review of the literature, Arch. Pathol. 96 (1973) 81–82.
[15] I.B. Kern, M.J. Harris, Congenital short bowel, Aust. N. Z. J. Surg. 42 (1973) 283–285.
[16] C. Nezelof, F. Jaubert, G. Lyon, Familial syndrome combining short small intestine, intestinal malrotation, pyloric hypertrophy and brain malformation. 3 anatomoclinical case reports, Ann. Anat. Pathol. (Paris) 21 (1976) 401–412.
[17] P. Royer, C. Ricour, C. Nihoul-Fekete, D. Pellerin, The familial syndrome of short small intestine with intestinal malrotation and hypertrophic stenosis of the pylorus in infants, Arch. Fr. Pediatr. 31 (1974) 223–229.
[18] C. Sansaricq, W.J. Chen, M. Manka, D. Davis, S. Snyderman, Familial congenital short small bowel with associated defects. A long-term survival, Clin. Pediatr. (Phila.) 23 (1984) 453–455.
[19] W.A. Huysman, D. Tibboel, J.H. Bergmeijer, J.C. Molenaar, Long-term survival of a patient with congenital short bowel and malrotation, J. Pediatr. Surg. 26 (1991) 103–105.
[20] J. Schalamon, P.H. Schober, P. Gallippi, L. Matthyssens, M.E. Hollwarth, Congenital short-bowel; a case study and review of the literature, Eur. J. Pediatr. Surg. 9 (1999) 248–250.
[21] A.I. De Backer, P.M. Parizel, A. De Schepper, W. Vaneerdeweg, A patient with congenital short small bowel associated with malrotation, J. Belge Radiol. 80 (1997) 71–72.
[22] K. Matter, M.S. Balda, Signalling to and from tight junctions, Nat. Rev. Mol. Cell Biol. 4 (2003) 225–236.
[23] B. Shehata, T. Chang, C. Greene, C. Steelman, M. McHugh, A. Zarroug, R. Ricketts, Gastric heterotopia with extensive involvement of the small intestine associated with congenital short bowel syndrome and intestinal malrotation, Fetal Pediatr. Pathol. 30 (2011) 60–63.
[24] K. Dumke, N. Schnoy, A rare case of a congenital short intestine, Z. Gastroenterol. 12 (1974) 321–325.
[25] I. Erez, O. Reish, M. Kovalivker, L. Lazar, A. Raz, S. Katz, Congenital short-bowel and malrotation: clinical presentation and outcome of six affected offspring in three related families, Eur. J. Pediatr. Surg. 11 (2001) 331–334.
[26] H.C. Peng, H.C. Chen, W.T. Chang, Congenital short small intestine: report of three cases, J. Formos. Med. Assoc. 92 (1993) 762–764.
[27] R.P. Kapur, S.P. Robertson, M.C. Hannibal, L.S. Finn, T. Morgan, M. van Kogelenberg, D.J. Loren, Diffuse abnormal layering of small intestinal smooth muscle is present in patients with FLNA mutations and x-linked intestinal pseudo-obstruction, Am. J. Surg. Pathol. 34 (2010) 1528–1543.
[28] G.I. Koffeman, W.G. van Gemert, E.K. George, R.A. Veenendaal, Classification, epidemiology and aetiology, Best Pract. Res. Clin. Gastroenterol. 17 (2003) 879–893.
[29] S.M. Chu, C.C. Luo, Y.H. Chou, J.B. Yen, Congenital short bowel syndrome with malrotation, Chang Gung Med. J. 27 (2004) 548–550.
[30] M. Hasosah, D.A. Lemberg, E. Skarsgard, R. Schreiber, Congenital short bowel syndrome: a case report and review of the literature, Can. J. Gastroenterol. 22 (2008) 71–74.
[31] T. Savin, N.A. Kurpios, A.E. Shyer, P. Florescu, H. Liang, L. Mahadevan, C.J. Tabin, On the growth and form of the gut, Nature 476 (2011) 57–62.
[32] B.M. Carlson, Human Embryology and Developmental Biology, Elsevier/Saunders, Philadelphia, Pa., 2014
[33] K.L. Moore, T.V.N. Persaud, The Developing Human: Clinically Oriented Embryology, Saunders, Philadelphia, Pa., 2003
[34] G.C. Schoenwolf, W.J. Larsen, Larsen's Human Embryology, Churchill Livingstone/Elsevier, Philadelphia, 2009.
[35] A.M. Goldstein, R.M. Hofstra, A.J. Burns, Building a brain in the gut: development of the enteric nervous system, Clin. Genet. 83 (2013) 307–316.
[36] P. De Santa Barbara, J. Williams, A.M. Goldstein, A.M. Doyle, C. Nielsen, S. Winfield, S. Faure, D.J. Roberts, Bone morphogenetic protein signaling pathway plays multiple roles during gastrointestinal tract development, Dev. Dyn. 234 (2005) 312–322.
[37] M. Ramalho-Santos, D.A. Melton, A.P. McMahon, Hedgehog signals regulate multiple aspects of gastrointestinal development, Development 127 (2000) 2763–2772.
[38] R. Aviram, I. Erez, T.Z. Dolfin, S. Katz, Y. Beyth, R. Tepper, Congenital short-bowel syndrome: prenatal sonographic findings of a fatal anomaly, J. Clin. Ultrasound 26 (1998) 106–108.
[39] R.N. Shawis, L. Rangecroft, R.C. Cook, D.C. Gough, Functional intestinal obstruction associated with malrotation and short small-bowel, J. Pediatr. Surg. 19 (1984) 172–173.
[40] C.F. Wei, C.T. Lai, Congenital short small intestine in siblings, Taiwan Yi Xue Hui Za Zhi 84 (1985) 620–624.
[41] E. Duveau, D. Bardot-Labbe, L. Larget-Piet, L. Coupris, J.L. Ginies, Congenital short bowel syndrome with intestinal malrotation: an unusual cause of chronic diarrhea, Gastroenterol. Clin. Biol. 24 (2000) 585–587.
[42] I.B. Kern, A. Leece, T. Bohane, Congenital short gut, malrotation, and dysmotility of the small bowel, J. Pediatr. Gastroenterol. Nutr. 11 (1990) 411–415.
[43] J.W. Hou, T.R. Wang, Amelia, dextrocardia, asplenia, and congenital short bowel in deleted ring chromosome 4, J. Med. Genet. 33 (1996) 879–881.
[44] L. Palle, B. Reddy, Case report: congenital short bowel syndrome, Indian J. Radiol. Imaging 20 (2010) 227–229.
[45] E. Raschperger, U. Engstrom, R.F. Pettersson, J. Fuxe, CLMP, a novel member of the CTX family and a new component of epithelial tight junctions, J. Biol. Chem. 279 (2004) 796–804.
[46] M.S. Balda, K. Matter, The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression, EMBO J. 19 (2000) 2024–2033.
[47] C.S. van der Werf, N.H. Hsiao, S. Conroy, J. Paredes, A.S. Ribeiro, Y. Sribudiani, R. Seruca, R.M. Hofstra, H. Westers, S.C. van Ijzendoorn, CLMP is essential for intestinal development, but does not play a key role in cellular processes involved in intestinal epithelial development, PLoS One 8 (2013) e54649.
[48] J.W. Fox, E.D. Lamperti, Y.Z. Eksioglu, S.E. Hong, Y. Feng, D.A. Graham, I.E. Scheffer, W.B. Dobyns, B.A. Hirsch, R.A. Radtke, S.F. Berkovic, P.R. Huttenlocher, C.A. Walsh, Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia, Neuron 21 (1998) 1315–1325.
[49] S.P. Robertson, S.R. Twigg, A.J. Sutherland-Smith, V. Biancalana, R.J. Gorlin, D. Horn, S.J. Kenwrick, C.A. Kim, E. Morava, R. Newbury-Ecob, K.H. Orstavik, O.W. Quarrell, C.E. Schwartz, D.J. Shears, M. Suri, J. Kendrick-Jones, A.O. Wilkie, O.P.-s.D.C.C. Group, Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans, Nat. Genet. 33 (2003) 487–491.
[50] J.A. Bernstein, D. Bernstein, U. Hehr, L. Hudgins, Familial cardiac valvulopathy due to filamin A mutation, Am. J. Med. Genet. A 155A (2011) 2236–2241.
[51] F. Kyndt, J.P. Gueffet, V. Probst, P. Jaafar, A. Legendre, F. Le Bouffant, C. Toquet, E. Roy, L. McGregor, S.A. Lynch, R. Newbury-Ecob, V. Tran, I. Young, J.N. Trochu, H. Le Marec, J.J. Schott, Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy, Circulation 115 (2007) 40–49.
[52] R. Oegema, J.M. Hulst, S.D. Theuns-Valks, L.M. van Unen, R. Schot, G.M. Mancini, M.E. Schipper, M.C. de Wit, B.J. Sibbles, I.F. de Coo, V. Nanninga, R.M. Hofstra, D.J. Halley, A.S. Brooks, Novel no-stop FLNA mutation causes multi-organ involvement in males, Am. J. Med. Genet. A 161A (2013) 2376–2384.
[53] A. Auricchio, V. Brancolini, G. Casari, P.J. Milla, V.V. Smith, M. Devoto, A. Ballabio, The locus for a novel syndromic form of neuronal intestinal pseudoobstruction maps to Xq28, Am. J. Hum. Genet. 58 (1996) 743–748.
[54] A. Gargiulo, R. Auricchio, M.V. Barone, G. Cotugno, W. Reardon, P.J. Milla, A. Ballabio, A. Ciccodicola, A. Auricchio, Filamin A is mutated in X-linked chronic idiopathic intestinal pseudo-obstruction with central nervous system involvement, Am. J. Hum. Genet. 80 (2007) 751–758.
[55] S.P. Robertson, Filamin A: phenotypic diversity, Curr. Opin. Genet. Dev. 15 (2005) 301–307.
[56] W. Deng, C. Lopez-Camacho, J.Y. Tang, D. Mendoza-Villanueva, A. Maya-Mendoza, D.A. Jackson, P. Shore, Cytoskeletal protein filamin A is a nucleolar protein that suppresses ribosomal RNA gene transcription, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 1524–1529.

[57] Y. Feng, M.H. Chen, I.P. Moskowitz, A.M. Mendonza, L. Vidali, F. Nakamura, D.J. Kwiatkowski, C.A. Walsh, Filamin A (FLNA) is required for cell–cell contact in vascular development and cardiac morphogenesis, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 19836–19841.
[58] M. Nishita, S.K. Yoo, A. Nomachi, S. Kani, N. Sougawa, Y. Ohta, S. Takada, A. Kikuchi, Y. Minami, Filopodia formation mediated by receptor tyrosine kinase Ror2 is required for Wnt5a-induced cell migration, J. Cell Biol. 175 (2006) 555–562.
[59] M. Yamada, J. Udagawa, A. Matsumoto, R. Hashimoto, T. Hatta, M. Nishita, Y. Minami, H. Otani, Ror2 is required for midgut elongation during mouse development, Dev. Dyn. 239 (2010) 941–953.
[60] B.E. Finley, J. Burlbaw, T.L. Bennett, L. Levitch, Delayed return of the fetal midgut to the abdomen resulting in volvulus, bowel obstruction, and gangrene of the small intestine, J. Ultrasound Med. 11 (1992) 233–235.
[61] G.E. Besner, G.D. Bates, C.P. Boesel, V. Singh, S.E. Welty, C.A. Corpron, Total absence of the small bowel in a premature neonate, Pediatr. Surg. Int. 21 (2005) 396–399.
[62] R. Guerrini, D. Mei, S. Sisodiya, F. Sicca, B. Harding, Y. Takahashi, T. Dorn, A. Yoshida, J. Campistol, G. Kramer, F. Moro, W.B. Dobyns, E. Parrini, Germline and mosaic mutations of FLN1 in men with periventricular heterotopia, Neurology 63 (2004) 51–56.
[63] A. Kakita, S. Hayashi, F. Moro, R. Guerrini, T. Ozawa, K. Ono, S. Kameyama, C.A. Walsh, H. Takahashi, Bilateral periventricular nodular heterotopia due to filamin 1 gene mutation: widespread glomeruloid microvascular anomaly and dysplastic cytoarchitecture in the cerebral cortex, Acta Neuropathol. 104 (2002) 649–657.
[64] F. Nakamura, T.M. Osborn, C.A. Hartemink, J.H. Hartwig, T.P. Stossel, Structural basis of filamin A functions, J. Cell Biol. 179 (2007) 1011–1025.
[65] H. Qiu, R. Nomiyama, K. Moriguchi, T. Fukada, K. Sugimoto, Identification of novel nuclear protein interactions with the N-terminal part of filamin A, Biosci. Biotechnol. Biochem. 75 (2011) 145–147.
[66] M.P. Playford, E. Nurminen, O.T. Pentikainen, S.L. Milgram, J.H. Hartwig, T.P. Stossel, F. Nakamura, Cystic fibrosis transmembrane conductance regulator interacts with multiple immunoglobulin domains of filamin A, J. Biol. Chem. 285 (2010) 17156–17165.
[67] L. Minsaas, J. Planaguma, M. Madziva, B.F. Krakstad, M. Masia-Balague, A.A. Katz, A.M. Aragay, Filamin a binds to CCR2B and regulates its internalization, PLoS One 5 (2010) e12212.
[68] A.W. Hart, J.E. Morgan, J. Schneider, K. West, L. McKie, S. Bhattacharya, I.J. Jackson, S.H. Cross, Cardiac malformations and midline skeletal defects in mice lacking filamin A, Hum. Mol. Genet. 15 (2006) 2457–2467.
[69] M.J. Geske, X. Zhang, K.K. Patel, D.M. Ornitz, T.S. Stappenbeck, Fgf9 signaling regulates small intestinal elongation and mesenchymal development, Development 135 (2008) 2959–2968.
[70] J. Mao, B.M. Kim, M. Rajurkar, R.A. Shivdasani, A.P. McMahon, Hedgehog signaling controls mesenchymal growth in the developing mammalian digestive tract, Development 137 (2010) 1721–1729.
[71] T.H. Kim, B.M. Kim, J. Mao, S. Rowan, R.A. Shivdasani, Endodermal hedgehog signals modulate Notch pathway activity in the developing digestive tract mesenchyme, Development 138 (2011) 3225–3233.
[72] S. Cervantes, T.P. Yamaguchi, M. Hebrok, Wnt5a is essential for intestinal elongation in mice, Dev. Biol. 326 (2009) 285–294.
[73] M. Matsuyama, S. Aizawa, A. Shimono, Sfrp controls apicobasal polarity and oriented cell division in developing gut epithelium, PLoS Genet. 5 (2009) e1000427.
[74] B. Hentsch, I. Lyons, R. Li, L. Hartley, T.J. Lints, J.M. Adams, R.P. Harvey, Hlx homeo box gene is essential for an inductive tissue interaction that drives expansion of embryonic liver and gut, Genes Dev. 10 (1996) 70–79.
[75] N. Itoh, D.M. Ornitz, Functional evolutionary history of the mouse Fgf gene family, Dev. Dyn. 237 (2008) 18–27.
[76] K.A. Waite, C. Eng, From developmental disorder to heritable cancer: it's all in the BMP/TGF-beta family, Nat. Rev. Genet. 4 (2003) 763–773.
[77] X.M. Zhang, M. Ramalho-Santos, A.P. McMahon, Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R asymmetry by the mouse node, Cell 105 (2001) 781–792.
[78] A.P. McMahon, P.W. Ingham, C.J. Tabin, Developmental roles and clinical significance of hedgehog signaling, Curr. Top. Dev. Biol. 53 (2003) 1–114.
[79] M.J. Bitgood, A.P. McMahon, Hedgehog and Bmp genes are coexpressed at many diverse sites of cell–cell interaction in the mouse embryo, Dev. Biol. 172 (1995) 126–138.
[80] N.A. Bhowmick, E.G. Neilson, H.L. Moses, Stromal fibroblasts in cancer initiation and progression, Nature 432 (2004) 332–337.
[81] P. de Santa Barbara, G.R. van den Brink, D.J. Roberts, Molecular etiology of gut malformations and diseases, Am. J. Med. Genet. 115 (2002) 221–230.
[82] B. D'Souza, L. Meloty-Kapella, G. Weinmaster, Canonical and non-canonical Notch ligands, Curr. Top. Dev. Biol. 92 (2010) 73–129.
[83] R. Kopan, M.X. Ilagan, The canonical Notch signaling pathway: unfolding the activation mechanism, Cell 137 (2009) 216–233.
[84] G.M. Collu, A. Hidalgo-Sastre, K. Brennan, Wnt-Notch signalling crosstalk in development and disease, Cell. Mol. Life Sci. 71 (2014) 3553–3567.
[85] P.D. Turnpenny, B. Alman, A.S. Cornier, P.F. Giampietro, A. Offiah, O. Tassy, O. Pourquie, K. Kusumi, S. Dunwoodie, Abnormal vertebral segmentation and the Notch signaling pathway in man, Dev. Dyn. 236 (2007) 1456–1474.
[86] U. Koch, F. Radtke, Notch and cancer: a double-edged sword, Cell. Mol. Life Sci. 64 (2007) 2746–2762.
[87] C. Crosnier, N. Vargesson, S. Gschmeissner, L. Ariza-McNaughton, A. Morrison, J. Lewis, Delta-Notch signalling controls commitment to a secretory fate in the zebrafish intestine, Development 132 (2005) 1093–1104.
[88] S. Fre, M. Huyghe, P. Mourikis, S. Robine, D. Louvard, S. Artavanis-Tsakonas, Notch signals control the fate of immature progenitor cells in the intestine, Nature 435 (2005) 964–968.
[89] W.H. Lien, E. Fuchs, Wnt some lose some: transcriptional governance of stem cells by Wnt/β-catenin signaling, Genes Dev. 28 (2014) 1517–1532.
[90] H. Clevers, R. Nusse, Wnt/β-catenin signaling and disease, Cell 149 (2012) 1192–1205.
[91] J.L. Green, S.G. Kuntz, P.W. Sternberg, Ror receptor tyrosine kinases: orphans no more, Trends Cell Biol. 18 (2008) 536–544.
[92] T. Reya, H. Clevers, Wnt signalling in stem cells and cancer, Nature 434 (2005) 843–850.
[93] E.R. Bakker, L. Raghoebir, P.F. Franken, W. Helvensteijn, L. van Gurp, F. Meijlink, M.A. van der Valk, R.J. Rottier, E.J. Kuipers, W. van Veelen, R. Smits, Induced Wnt5a expression perturbs embryonic outgrowth and intestinal elongation, but is well-tolerated in adult mice, Dev. Biol. 369 (2012) 91–100.
[94] F. He, W. Xiong, X. Yu, R. Espinoza-Lewis, C. Liu, S. Gu, M. Nishita, K. Suzuki, G. Yamada, Y. Minami, Y. Chen, Wnt5a regulates directional cell migration and cell proliferation via Ror2-mediated noncanonical pathway in mammalian palate development, Development 135 (2008) 3871–3879.
[95] D. Qian, C. Jones, A. Rzadzinska, S. Mark, X. Zhang, K.P. Steel, X. Dai, P. Chen, Wnt5a functions in planar cell polarity regulation in mice, Dev. Biol. 306 (2007) 121–133.
[96] P. Bovolenta, P. Esteve, J.M. Ruiz, E. Cisneros, J. Lopez-Rios, Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease, J. Cell Sci. 121 (2008) 737–746.
[97] W. Satoh, M. Matsuyama, H. Takemura, S. Aizawa, A. Shimono, Sfrp1, Sfrp2, and Sfrp5 regulate the Wnt/beta-catenin and the planar cell polarity pathways during early trunk formation in mouse, Genesis 46 (2008) 92–103.
[98] M.K. Dush, N.M. Nascone-Yoder, Jun N-terminal kinase maintains tissue integrity during cell rearrangement in the gut, Development 140 (2013) 1457–1466.
[99] J.D. Allen, T. Lints, N.A. Jenkins, N.G. Copeland, A. Strasser, R.P. Harvey, J.M. Adams, Novel murine homeo box gene on chromosome 1 expressed in specific hematopoietic lineages and during embryogenesis, Genes Dev. 5 (1991) 509–520.
[100] T.J. Lints, L. Hartley, L.M. Parsons, R.P. Harvey, Mesoderm-specific expression of the divergent homeobox gene Hlx during murine embryogenesis, Dev. Dyn. 205 (1996) 457–470.